PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below
International application No. PCT/US2004/040289	International filing date *(day/month/year)* 01.12.2004
Priority date *(day/month/year)* 01.12.2003	
International Patent Classification (IPC) or both national classification and IPC C12Q1/68	
Applicant EPIGENOMICS AG	

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2004/040289

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1, 3-47 partially

because:

☒ the said international application, or the said claims Nos. 5, 14, 33 relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 1, 38 are so unclear that no meaningful opinion could be formed *(specify)*:

see separate sheet

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 1, 3-47 partially

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form
- ☐ has not been furnished
- ☐ does not comply with the standard

the computer readable form
- ☐ has not been furnished
- ☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☒ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. Invention 1: claim 2 completely and claims 1, 4, 5-47 partially

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	33-37
	No:	Claims	1, 2, 4-32, 38-47
Inventive step (IS)	Yes:	Claims	33-37
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1, 2, 4, 6-13, 15-32, 34-47
	No:	Claims	

2. Citations and explanations

   see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)

   and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

   see form 210

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 5, 14 and 33 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).
For the assessment of the present claims 5, 14 and 33 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Claim 1 refers to the use of a combination of the given genes in a method for the detection and/or differentiation between prostate cell proliferative disorders. Said claim does not meet the requirements of Art. 6 PCT in that it encompasses a huge number of embodiments (all the different combinations of any of two, three, four, etc... from the total in the group) such that a meaningful search is impossible. The description provides examples in which SEQ ID NO:1023 has been used in combination with other two or three genes. The search has been therefore been carried out for the specific combinations disclosed in Table 22, page 136 and Table 23, page 137 that concern SEQ ID NO:1023.

Claim 38 is unclear (Art. 6 PCT) to the extent that no meaningful search is possible across the entire scope of the claim. Said claim relates to a nucleic acid molecule that is not defined in terms of its technical features, but only with respect to a vaguely defined chemical treatment. Furthermore the term "derived from" is vague and open to interpretation. The only sequences derived from SEQ ID NO:1023 that are clear from the application are those defined by SEQ ID NOs: 1041, 1042, 1065 and 1066, these being possible results of a bisulfite treatment. Therefore the search has been restricted to these specific sequences.

According to Table 26 in pages 140-142 of the description, the four sequences post-treatment (methylated, unmethylated and sense and antisense of each) that correspond to SEQ ID NO:1023 are SEQ ID NOs:1036, 1042, 1065, 1066. However the analysis of the sequences filed electronically has revealed that SEQ ID NO:1036 does not relate to SEQ ID NO:1023. Therefore the search has been carried out for SEQ ID NOs:1041, 1042, 1065, 1066 as sequences related to SEQ ID NO:1023, instead of SEQ ID NOs:1036, 1042, 1065, 1066 as indicated in Table 26.

Re Item IV

Lack of unity of invention

As indicated in the International Search Report (ISR) established by this authority the present application does not fulfill the requirements of Rule 13.1 PCT (see reasoning in the ISR) and the applicant was therefore invited to pay additional fees.
No additional fees were paid and therefore only the subject-matter concerning the first invention, i.e. the subject-matter of claim 2 completely and claims 1, 4, 5-47 partially (when concerning SEQ ID NO:1023 and its related sequences) will be examined according to Rule 68.5 PCT.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: WO 02/18632 A (EPIGENOMICS AG; OLEK, ALEXANDER; PIEPENBROCK, CHRISTIAN; BERLIN, KURT;) 7 March 2002 (2002-03-07).

D2: WO 02/103042 A (EPIGENOMICS AG) 27 December 2002 (2002-12-27).

D3: YAMADA YASUSHI ET AL: "Aberrant methylation of the vascular endothelial growth

factor receptor-1 gene in prostate cancer." CANCER SCIENCE, vol. 94, no. 6, June 2003 (2003-06), pages 536-539.

Novelty (Art 33(2) PCT)

Document D1 discloses a method for determining the degree of methylation of a particular cytosine in a motif 5'-CpG-3', present in a genomic sample of DNA. The sample is treated chemically to convert cytosine, but not methylated C, to uracil and hybridized to oligonucleotides and/or peptide-nucleic acid (PNA) oligomers whose degree of hybridization determine the degree of methylation.
The oligonucleotides identified by SEQ ID NOs:29231 and 29232 have a length of 579nt and have 99.14% identity over the 579nt with SEQ ID NO:1041 of the present application.
The oligonucleotides identified by SEQ ID NOs:29229 and 29230 have a length of 579nt and have 98.96% identity over the 579nt with SEQ ID NO:1042 of the present application.
As SEQ ID Nos:1041 and 1042 correspond to the "treated" sequences derived from SEQ ID NO:1023, the subject-matter of claims 38-44 is not new.
For claims directed to a physical entity, non-distinctive characteristics of a particular intended use, are disregarded in determining novelty of the subject matter (see PCT International Search and Preliminary Examination Guidelines, Chapter 12, paragraph 12.05). Therefore the intended use of the kit of claim 45 "useful for detecting or distinguishing between or among prostate cell proliferative disorders of a subject" is disregarded.
As D1 also discloses a kit comprising bisulfite and the oligonucleotides identified by SEQ ID Nos: 29229, 29230, 29231 and 29232, the subject-matter of claims 45, 46 is not new.

Document D2 discloses a method for determining genetic and/or epigenetic parameters for the classification, differentiation and/or diagnosis of prostate tumors. The method comprises analyzing cytosine methylation by obtaining a biological sample containing genomic DNA; extracting the genomic DNA; in the genomic DNA sample, cytosine bases that are unmethylated at the 5-position are converted by chemical treatment, to uracil or another base, which is dissimilar to cytosine in terms of hybridization behavior; and amplifying at least one fragment of the chemically pre-treated genomic DNA using sets of primer oligonucleotides and a polymerase, where the genomic CpG sequences are located

within the chemically treated genomic sequences identified by SEQ ID NOs: 1-112.
Fragments of at least 9 contiguous nucleotides from the chemically treated DNA genomic sequences SEQ ID NOs: 1-112 are used in the method.
SEQ ID NO:17 of D2 has 22 contiguous nucleotides that are identical to SEQ ID NO:1041 and SEQ ID NO:1065 of the present application (nucleotides 12583-12605 of SEQ ID NO:17 are identical to nucleotides 1-22 of SEQ ID NO:1041 and SEQ ID NO:1065).
SEQ ID NO:51 of D2 has 39 contiguous nucleotides that are identical to SEQ ID NO:1042 and SEQ ID NO:1066 of the present application (nucleotides 8218-8256 of SEQ ID NO:51 are identical to nucleotides 6198-6236 of SEQ ID NO:1042 and SEQ ID NO:1066).
Therefore D2 discloses a method for the detection and/or differentiation between prostate cell proliferative disorders that comprises the use of a contiguous sequence of at least 9 nucleotides that is complementary to or hybridizes to SEQ ID NO: 1041 and SEQ ID NO: 1042.
The subject-matter of claims 1, 2, 4-32, 47 is therefore not new.

Inventive step (Art 33(3) PCT)

Document D3 discloses a method for detecting prostate cancer by determining the aberrant methylation of the vascular endothelial growth factor receptor-1 gene. Said method is a combined bisulfite restriction analysis (COBRA) that comprises the use of methylation-sensitive restriction enzymes.
The differene between D3 and the subject-matter of claim 33 is that, whereas in D3 the method determines the methylation of the VEGFR-1 gene, in claim 33 the method determines the methylation of SEQ ID NO:1023.
Therefore in the light of D3 the problem solved by the subject-matter of claim 33 is the provision of an alternative method for detecting prostate cancer; the solution being a method that determines the methylation of SEQ ID NO:1023 by using methylation-sensitive restriction enzymes.
Said solution is regarded as inventive because there was no indication in the prior art that the methylation pattern of SEQ ID NO:1023 could be related to prostate cell proliferative disorders.
The subject-matter of claims 33-37 is therefore inventive.

Re Item VIII

Certain observations on the international application

Claims 1, 2, 4, 5-8 do not meet the requirements of Art. 6 PCT in that the method is described by the result to be achieved, i.e. detecting in a sample containing all o part of SEQ ID NO:1023 the presence of methylated and non-methylated CpG dinucleotides, but it does not include the technical features that are essential to the method, i.e. specific oligonucleotides that distinguish between methylated and non-methylated CpG or the treatment with methylation-sensitive restriction enzymes. Where patentability depends on a technical effect the claims must be so drafted as to include all the technical features of the invention which are essential for the technical effect.

Present claims 5 and 33 do not meet the requirements of Rule 6.1(a) PCT because they have been drafted as separate independent claims, although they relate to the same subject-matter and differ from each other only with regard to the definition of the subject-matter for which protection is sought and in respect of the terminology used for the features of that subject-matter. The aforementioned claims lack therefore conciseness.

Claim 38 is unclear (Art. 6 PCT) because it relates to a nucleic acid molecule that is not defined in terms of its technical features, but only with respect to a vaguely defined chemical treatment.

Claim 42 is unclear (Art. 6 PCT) in that it reads "to a treated genomic DNA sequence selected from the group" but refers to the SEQ ID NOs that correspond to the "untreated" genomic DNA sequences.

Table 26 in pages 140-142 of the description contains an error; in page 141 SEQ ID NO:1032 corresponds, according to this table, both to the antisense methylated sequence derived from SEQ ID NO:1018 and to the sense methylated sequence derived from SEQ ID NO:1019. As consequence of this error the numbering SEQ ID NO:1033-1041 that follows in the sense methylated column is wrong and those sequences do not correspond

to the genomic sequences specified in the first column.